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Effect of *i*NOX Inhibitor Aminoguanidine Hemisulfate on Amikacin Induced Consequences on Anti-oxidant Stress Markers in Wistar Rat

Makhmoor Ahmad[1], Shahid Prawez[2], Nasir Manzoor[1], Mudasir Sultana[1], Rajinder Raina[1], Mohd Yousuf Dar[3*] and Nrip Kishore Pankaj[1]

[1]Division of Veterinary Pharmacology and Toxicology, F.V.Sc. & A.H., SKUAST-J, Jammu and Kashmir, INDIA
[2]Division of Veterinary Pharmacology and Toxicology, FVAS-IAS, BHU, Varanasi, Uttar Pradesh, INDIA
[3]Division of Veterinary Anatomy, COVAS, SVPUAT, Meerut,Utter Pradesh, INDIA

**Corresponding author: M Yousuf; Email: yousufdar8@gmail.com*

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ABSTRACT

The present study was aimed to investigate the effect of aminoguanidine-hemisulphate on amikacin induced oxidative stress in wistar rats of both the sexes. Twenty-four healthy wistar rats were randomly divided into 4 groups. The rats of group-I served as control to which normal saline solution as vehicle was administered inperitoneally once daily for 28 days as sub-acute study period. The rats of group-II and group-III were treated with amikacin (15mg/Kg) and aminoguanidine-hemisulphate (20mg/Kg) daily for 28 days intra-peritoneally, respectively The rats of group-IV were treated with both amikacin and aminoguanidine-hemisulphate at their respective doses and routes once daily for 28 days. The activity of antioxidant stress biomarkers namely SOD, GPx, CAT and GSH levels were significantly decreases on days 15 and 28 after amikacin treatment as compared to control. The oxidative damage was protected in aminoguanidine-hemisulphate treated rats of group-IV as indicated by a significant increase in the activity of SOD, GPx, CAT and GSH-level as compared to amikacin treated rats. The present study suggests that amikacin has the potential of inducing oxidative stress as evidenced by alterations in antioxidant enzymes and GSH. However, Aminoguanidine-hemisulphate has ameliorative effect in normalizing the altered values of various oxidative stress biomarkers.

Keywords: Amikicin, Aminoguanidine, oxidative biomarkers, wistar rats.

Amikacin can also be used in combination with beta-lactam-antibiotics to produce synergistic-effect and also broaden the activity against both gram positive and gram negative bacteria (Sandhu *et al.,* 2007). It has potential to induce the lipid peroxidation that restricts amikacin therapeutic uses. Its nephrotoxicity and ototoxicity has widely guided attempts to rationalize the drug dosage strategy (Barclay and Begg, 1994). It produces free-radicals/reactive oxygen species (ROS) which participate in the patho-physiology of amikacin-induced-nephrotoxicity (Parlakpinar *et al.,* 2004). Aminoguanidine-hemisulfate is an effective antioxidant (Ihm *et al*., 1999) and a free radical scavenger (Szabo *et al*., 1997; Parlakpinar *et al*., 2004). It inhibits inducible nitric oxide synthase (*i*NOS) in a selective and competitive manner, leading to decreased generation of nitric-oxide (Misko *et al*., 1993) and free-radicals. Aminoguanidine is a nucleophilic reagent that can inhibit the formation of advanced glycation end products by reacting with reactive carbonyl groups of proteins to form relatively non-toxic adducts. This way, it stops the aging of the body and prevents thickening of the arteries, senile cataracts, age-related yellowing and toughening of the skin, some cancers, and damage to the immune system (Li *et al*., 1996). Therefore, present study was an attempt to study oxidative damage after amikacin administration and also to evaluate the protective role of aminoguanidine-hemisulfate in controlling such damage in wistar rats.

MATERIALS AND METHODS

Experimental animals

In the present study twenty-four healthy wistar rats were

procured from IIM, RRL, Jammu. After environmental acclimatization, rats of about 150-200g were randomly divided into four groups comprising six animals in each group. The rats of group-I served as control to which normal saline solution as vehicle was administered peritoneally once daily for 28 days as sub-acute study period. The rats of group-II and group-III were treated with amikacin and aminoguanidine-hemisulphate at the dose rate of 15mg/kg and 20mg/Kg body weight once daily for 28 days intra-peritoneally, respectively. The rats of group-IV were treated with both amikacin and aminoguanidine-hemisulphate at their respective doses and routes once daily for 28 days.

Sample collection

The blood samples of about 2-3ml were collected from retro-orbital sinus of rats on day zero, 15th and 29th of experimental period using capillary-tubes in test tubes containing anticoagulant heparin (10 IU/ml). However, anticoagulant sodium EDTA was used for the haematological parameters. The blood samples were centrifuged at 3000rpm for 15 min to harvest the plasma and stored in clean sterile tubes at -20ºC till further analysis. The erythrocyte sediment left over after separating the plasma was diluted with normal saline solution in the ratio of 1:1 (v/v) and thoroughly mixed. The diluted erythrocytes solution was centrifuged and supernatant with buffy-coat discarded. The same protocol was further repeated thrice with NSS in same proportion. After final washing, phosphate buffer solution (pH 7.4, 0.1M) was taken as diluent to make 1% (100µl washed RBC + 9.9 ml PBS) and 33% hemolysate (330µl washed RBC + 670µl PBS). The hemolysate-1% used as sample to estimate catalase and superoxide-dismutase (Marklund and Marklund, 1974), whereas, hemolysate-33% for the lipid-peroxidation (Shafiq-ul-Rehman, 1984) estimation. The activity of Glutathione peroxidase (GSH-Px), Glutathione-S-transferase (GST) and catalase (CAT) were assayed as per method described by Hafeman *et al.,* 1974, Habig *et al.,* 1974 and Aebi, 1983, respectively.

Statistical analysis

The data collected during the experiment was subjected to analysis of variance under completely randomized design (CRD) and the level of significance was tested using Duncan Multiple Range Test (Duncan, 1955) at 5% (P< 0.05) level of significance.

RESULTS AND DISCUSSION

A significantly decrease of SOD activity was observed on day 15 and 29 in amikacin treated rats/group-II, as compared to control rats/group-I (Table 1). Similar decrease in SOD activity was reported in amikacin treated rats (Dwivedi *et al*., 2009; Singhal and Prajapati, 2011). The decrease in activity because of amikacin induced generation of reactive oxygen species that leads to the exhaustion and overutilization of SOD by free radicals. Aminoguanidine treatment on day 15 and 29 produces a significant increase in SOD activity in rats of group-III as compared to control rats. This increase in SOD activity may be because of NOS inhibitor, amonoguanidine free radical scavenging property (Mansour *et al*., 2002; Al-Majed, 2004). In amikacin and aminoguanidine co-adminstered rats/group-IV, on day 15 and 29, a significant increase in SOD-activity was found as compared to amikacin treated rats/group-II. Abo-Salem, (2012) reported an increase in SOD activity was found in doxorubicin and aminoguanidine co-administered group compared to doxorubicin administered group. It may be due to as aminoguanidine act as reactive oxygen species scavenger, therefore reducing the reactive oxygen species generation which helps to increase the SOD activity found in co-administered group-IV (Mansour *et al*., 2002**;** Al-Majed, 2004).

Table 1: Effect of aminoguanidine hemisulfate on plasma Superoxide Dismutase (U/mg protein) activity in amikacin treated wistar rats

Treatments groups	Treatment period		
	Day zero (n=6)	Day fifteen (n=6)	Day twenty-nine (n=6)
Group-I (Control)	61.68 ± 1.89^{aA}	61.81 ± 1.31^{aB}	59.87 ± 1.20^{aB}
Group-II (Amikacin)	61.16 ± 1.88^{aA}	44.47 ± 0.98^{bD}	43.70 ± 0.85^{bD}
Group-III (Aminoguanidine)	60.99 ± 1.01^{bA}	66.80 ± 0.83^{aA}	69.30 ± 0.79^{aA}
Group-IV (Amikacin +Aminoguanidine)	60.34 ± 1.22^{aA}	49.39 ± 1.26^{bC}	53.17 ± 1.30^{bC}

Values are in Mean ± SE, Similar superscript do not differ significantly at 5% (P<0.05); Capital superscripts represent significance between the groups; Small superscripts represent significance within the groups.

Daily administration of amikacin in rats of group-II, significantly decreases the catalase activity as compared to control rats (Table 2). Similar significant decrease in catalase activity was reported in amikacin treated rats (Singhal and Prajapati, 2011). This may be due to exhaustion and overutilization of catalase to nullify the effect of free radicals generated in amikacin treated group. However, on day 15 and 29, a significant increase in catalase activity was observed in aminoguanidine treated rats of group-III as compared to control rats at their respective time interval. On day 15 and 29 in amikacin and aminoguanidine co-adminstered rats/group-IV, a significant increase in catalase activity was found as compared to amikacin treated rats/group-II in respective time period. Similar increase in catalase activity has also been reported by Abraham *et al.* (2009) and Al-Majed, (2004) in aminoguanidine treated rats, inhibiting iNOS and scavenging free radicals.

Table 2: Effect of aminoguanidine hemisulfate on plasma catalase activity (µmole H_2O_2 decomposed/min/mg protein) activity in amikacin treated wistar rats

Treatment groups	Treatment period		
	Day zero (n=6)	Day fifteen (n=6)	Day twenty-nine (n=6)
Group-I (control)	50.85 ± 0.90^{aA}	51.55 ± 1.01^{aB}	51.89 ± 1.29^{aB}
Group-II (amikacin)	49.21 ± 1.03^{aA}	35.05 ± 1.98^{bD}	30.43 ± 1.39^{cD}
Group-III (aminoguanidine)	50.05 ± 0.47^{cA}	57.60 ± 1.13^{bA}	61.97 ± 0.52^{aA}
Group-IV (amikacin+ aminoguanidine)	50.42 ± 1.73^{aA}	46.81 ± 0.80^{aC}	48.23 ± 0.91^{aC}

Values are in Mean ± SE, Similar superscript do not differ significantly at 5% (P<0.05); Capital superscripts represent significance between the groups; Small superscripts represent significance within the groups.

Daily administration of amikacin in rats of group-II significantly decreases the GPx activity as compared to control rats (Table 3). Similar significant decrease in GPx activity was reported (Yazar *et al.,* 2003) after amikacin treatment. Glutathione-peroxidase is a selenium containing enzyme which reduces hydrogen peroxide utilizing GSH and thereby serves as an alternative means of detoxifying activated oxygen. Such decrease in GPx activity is through free radicals induced by amikacin and singlet oxygen which directly inactivates GPx activities.

However, amikacin plus aminoguanidine treated group-IV, significantly increases the blood GPx level as compared to amikacin treated rats of group-I. Similar increase in GPx activity was reported by Yildirim *et al*. (2004) in aminoguanidine treated rats. Aminoguanidine, a free radical scavenger and therefore in amikacin plus aminoguanidine treated rats, less free radicals formation was found. The less free radicals formation correlate with less GPx utilization to combat the free radicals loads indicated by high plasma GPx level in amikacin plus aminoguanidine treated groups.

Table 3: Effect of aminoguanidine hemisulfate on plasma glutathione peroxidase activity (U/mg protein) in amikacin treated wistar rats

Treatments groups	Treatment-period		
	Day zero (n=6)	Day fifteen (n=6)	Day twenty-nine(n=6)
Group-I (Control)	11.42 ± 0.27^{aA}	11.84 ± 0.44^{aB}	11.21 ± 0.46^{aC}
Group-II (Amikacin)	11.24 ± 0.34^{aA}	9.43 ± 0.24^{bC}	7.55 ± 0.28^{cD}
Group-III (Aminoguanidine)	11.23 ± 0.35^{bA}	13.85 ± 0.37^{aA}	13.95 ± 0.40^{aA}
Group-IV (Amikacin+ Aminoguanidine)	11.41 ± 0.40^{bA}	12.70 ± 0.82^{aB}	12.68 ± 0.22^{aB}

Values are in Mean ± SE, Similar superscript do not differ significantly at 5% (P<0.05); Capital superscripts represent significance between the groups; Small superscripts represent significance within the groups.

A significant decrease in plasma glutathione level was observed in amikacin treated rats of group-I as compared to control rats (Table. 4). Similar significant decrease in glutathione was also reported in rats (Singhal and Prajapati, 2011) in amikacin treated rats. It may be due to excessive production of hydrogen peroxide after amikacin administration which inhibits the synthesis of phospholipases A2 and glutathione in rats. The ratio of free radical generating and free radical scavenging enzymes get disturbed leading to disruption in signal transduction pathway and increased the cellular permeability.

Amikacin plus aminoguanidine treated rats of group-IV, a significant increase in plasma glutathione level was found as compared to amikacin treated rats of group-II. Similar increase in glutathione was also reported in doxorubicin-induced nephropathic rats after administration of aminoguanidine (Abo-Salem, 2012). It may be due to ameliorative effect of aminoguanidine on amikacin-induced oxidative stress by inhibiting iNOS pathway and therefore, the decreased production of hydrogen peroxide.

Table 4: Effect of aminoguanidine hemisulfate on plasma Glutathione levels (nmol/ml) in amikacin treated wistar rats

Treatment groups	Treatment-period		
	Day zero (n=6)	Day fifteen (n=6)	Day twenty-nine (n=6)
Group-I (control)	51.68 ± 1.08^{aA}	51.31 ± 0.88^{aB}	52.03 ± 0.80^{aB}
Group-II (Amikacin)	52.55 ± 0.28^{aA}	44.25 ± 0.56^{bC}	42.41 ± 0.60^{cC}
Group-III (Aminoguanidine)	52.93 ± 0.74^{cA}	55.33 ± 0.63^{bA}	57.54 ± 0.37^{aA}
Group-IV (Amikacin+ Aminoguanidine)	52.20 ± 0.86^{aA}	50.67 ± 0.97^{aB}	50.08 ± 1.06^{aB}

Values are in Mean ± SE, Similar superscript do not differ significantly at 5% ($P<0.05$); Capital superscripts represent significance between the groups; Small superscripts represent significance within the groups

CONCLUSION

The study showed that amikacin has the potential of inducing oxidative stress as evidenced by alterations in antioxidant enzymes and GSH. However, Aminoguanidine-hemisulphate has ameliorative effect in normalizing the altered values of various oxidative stress biomarkers.

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